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THE MUTAGENIC POTENTIAL OF: n-hexyl-2-oxazolidone

LEONARD J. SAUERS, BA, SP5
FREDDICA R. PULLIAM, BS, SSG
and
JOHN T. FRUIN, DVM, PhD, LTC VC

TOXICOLOGY SERVICES GROUP,
DIVISION OF RESEARCH SUPPORT

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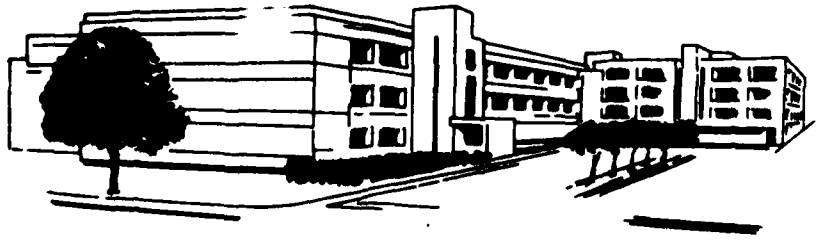

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J. W. H. Marshall 17 July 91

(Signature and date)

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

It has been shown that, with the Ames Assay, n-hexyl-2-oxazolidone is mutagenic to Salmonella strain TA 98 in the range of 4×10^{-5} to 3.2×10^{-7} ml/plate. Since these same results were not observed for any of the other strains, it was concluded that the test substance functions as a weak frameshift mutagen.

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ABSTRACT

It has been shown that, with the Ames Assay, n-hexyl-2-oxazolidone is mutagenic to Salmonella strain TA 98 in the range of 4×10^{-5} to 3.2×10^{-7} ml/plate doses. Since these same results were not observed for any of the other tester strains, it was concluded that the test substance functions as a weak frameshift mutagen.

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PREFACE

AMES ASSAY REPORT: n-hexyl-2-oxazolidone

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Division of Cutaneous Hazards
Letterman Army Institute of Research

PROJECT: More Effective Topical Repellents Against Disease Bearing Mosquitoes 3M62272A810

GLP STUDY NUMBER: 80007

STUDY DIRECTOR: LTC John Fruin, D.V.M., PhD
PRINCIPAL INVESTIGATOR: SSG Freddica R. Pulliam, BS

RAW DATA: A copy of the final report, study protocol, and retired SOP will be retained in the LAIR Archives. Test compounds were provided by sponsor. Chemical, analytical, stability, purity, etc. data are available from sponsor.

PURPOSE: To determine the mutagenic potential of n-hexyl-2-oxazolidone by using the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used.

ACKNOWLEDGMENT

The authors wish to thank SP5 Robert Summers for his assistance in performing the research.

Signatures of Principal Scientists Involved
In The Study

We, the undersigned, believe the study, GLP number 80007, described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Environmental Protection Agency.

Freddica R. Pulliam 16 Jul 81

FREDDICA R. PULLIAM /DATE
SSG, BS
Principal Investigator

John T. Fruin 17 Jul 81

JOHN T. FRUIN, DVM, PhD /DATE
LTC, VC
Study Director

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

8 January 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 80007 the following inspections were made:

9 June 1980
7 July 1980

Findings were reported to the Study Director and laboratory management on 7 August 1980. Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 1980 and October 1980 reports to management and the Study Director.

JOHN L. SZUREK
MAJ, MS
Quality Assurance Officer

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Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to *in vivo* tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair

mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous

revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS

Two separate Ames Tests were conducted on n-hexyl-2-oxazolidone, 20 May 80 and 27 May 80. Experimental error caused contamination of the top agar and invalidation of the results in the assay of 20 May 80. This was determined by extraneous growth on the minimum glucose agar plates containing only top agar and no test organism (Table 1A). The data that were obtained showed that the spontaneous revertant rate for TA 98 and TA 1538 without activation was below that suggested by Ames et al (2) (Table 1A). It should be noted that spontaneous reversion values below that suggested by Ames et al (2), are indicative of high quality water, materials, techniques, etc., and present no problem in the assay. Counts higher than those suggested by Ames et al (2) are indicators of serious assay performance proplems. The assay on 27 May 80 also showed a spontaneous reversion rate below that suggested by Ames et al (2) for TA 98 with activation and TA 1535 with and without activation (Table 1B). Below expected values were also seen for

nonactivated TA 1538. In this assay, the lawns for TA 98 and 1538 were uneven in the diluent controls (Table 1B). On 20 May 1980, TA 98 and TA 1538 showed unexpected results to positive control DMBA (Table 2A). On 27 May 1980, TA 1538 also did not react as expected to the positive control chemical dimethyl-benzanthracene (DMBA) (Table 2B). During the toxicity level determination, 10 fold dilutions were made from 0.1 ml/plate to $1x10^{-4}$ ml/plate. Quality control data for this part of the assay are shown in Table 3. The value determined as the sublethal dose was $1x10^{-3}$ ml of n-hexyl-2-oxazolidone /plate (Table 4).

DISCUSSION

In interpreting results, Ames et al (2) state that a substance is mutagenic if it yields twice the number of revertants experimentally compared to the number which occurs spontaneously. Data collected for the assay of 20 May 1980 are included but disregarded due to contamination (Table 5A). For the assay run on 27 May 1980, mutagenic activity was observed for both activated and nonactivated TA 98 at the $4x10^{-5}$, $8x10^{-6}$, $1.6x10^{-6}$ and $3.2x10^{-7}$ ml/plate concentrations. TA 1535 was genetically mutated at $3.2x10^{-7}$ ml/plate concentrations, both with and without activation. TA 1535 was mutated when activated at the $2x10^{-4}$, $8x10^{-6}$, and $1.6x10^{-6}$ ml/plate levels. TA 1537 and TA 1538 showed mutagenic effects only in the absence of S-9 at the $4x10^{-5}$ ml/plate and the $3.2x10^{-7}$ ml/plate levels (Table 5B). In addition to declaring the substance as a potential mutagen based on Ames criteria, the validity of our decision can be substantiated by using the MUTAR Ratio. The MUTAR values were calculated for all our data and are assembled in Table 6. The MUTAR values for nonactivated TA 98 are between 1.5 and 2.5 for dose levels $4x10^{-4}$ through $3.2x10^{-7}$ ml/plate. The substance has mutagenic properties for activated TA 98 at the same dose levels, with 95% probability, these MUTAR values are well above the 2.5 limit. All other strains in the assay demonstrate MUTAR values below 1.5; MUTAR values were not calculated for 20 May 1980 due to contamination.

CONCLUSION

The data indicate that, with the Ames Assay, n-hexyl-2-oxazolidone is mutagenic to TA 98 in the range of $4x10^{-5}$ to $3.2x10^{-7}$ ml/plate due to doubling of the spontaneous reversion rate and an obvious dose response. The results for TA 100, TA 1535, TA 1537 and TA 1538 did not demonstrate mutagenicity or dose response. The test substance probably functions as a weak frameshift mutagen. When n-hexyl-2-oxazolidone is activated, there is a 95% probability of mutagencity using the MUTAR table. The data used are those obtained on 27 May 1980.

RECOMMENDATION

The Ames Assay has demonstrated approximately 90% accuracy in predicting that mutagenic compounds are carcinogenic. It is equally as accurate in predicting that non-mutagenic compounds are non-carcinogenic. We recommend that, unless n-hexyl-2-oxazolidone shows potential insect repellent properties far above other compounds, it should not be subjected to further evaluation.

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LIST OF TABLES

APPENDIX

TABLE - 1A

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

20 May 80

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet	Sterility Control (e)
TA 98	+	+	+	13mm	NT
TA 100	GROWTH *	+	GROWTH **	14.5mm	NT
TA 1535	+	NA	+	13mm	NT
TA 1537	+	32mm	+	13mm	NT
TA 1538	+	NA	+	13mm	NT
WT	GROWTH	NA	GROWTH	NA	NA

QUALITY CONTROL (e) * 3 colonies "Biotin only" plate
** sparse growth on irradiated side of plate

His-Bio mix Initial: Contam. End: Contamination Test Compound 1: Contamination

Top Agar Initial: Contam. End: Contamination Test Compound 2: NA

S - 9 Initial: + End: + Test Compound 3: NA

Diluent: Contamination Nutrient Broth: + Test Compound 4: NA

MGA Plate w/ bacteria: + MGA Plate: + Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
TA 98	40	30-50	17	15	12	15	33	40	43	39
TA 100	160	120-200	129	139	139	136	157	118	117	131
TA 1535	20	10-35	14	22	17	18	10	20	13	14
TA 1537	7	3-15	4	6	5	5	8	11	10	10
TA 1538	25	15-35	11	15	10	12	14	28	15	19

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: F. Pulliam Date: 13 May 80

Test Read By: F. Pulliam Date: 20 May 80

TABLE - 1B

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

27 May 80

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet	Sterility Control (e)
TA 98	+	+	+	14mm	NG
TA 100	+	+	+	14.6mm	NG
TA 1535	+	NA	+	17mm	NG
TA 1537	+	26mm	+	17mm	NG
TA 1538	+	NA	+	18.7mm	NG
WT	GROWTH	NA	GROWTH	NA	NA

QUALITY CONTROL (e)

His-Bio mix Initial: + End: + Test Compound 1: NG

Top Agar Initial: + End: + Test Compound 2: NA

S - 9 Initial: + End: + Test Compound 3: NA

Diluent: 98 A & 1538 A = lawns uneven Nutrient Broth: + Test Cpmpound 4: NA

MGA Plate w/ bacteria: + MGA Plate: + Test Compound 5: NA

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type.

Spontaneous Revertants (1)

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
TA 98	40	30-50	23	32	35	30	26	18	25	23
TA 100	160	120-200	140	128	122	130	136	135	126	132
TA 1535	20	10-35	5	6	6	6	9	3	7	6
TA 1537	7	3-15	2	4	3	3	5	7	7	6
TA 1538	25	15-35	10	7	4	7	13	17	20	17

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: F. Pulliam Date: 25 May 80

Test Read By: F. Pulliam Date: 27 May 80

TABLE 2-A

POSITIVE CONTROL REVERTANT RATE

Date	Strain	Spontaneous Rev		AF	MNNG	BP	DMBA	Re-sponse (a)	Unit
		S-9	No S-9	S-9	No S-9	S-9	S-9		
20 May	TA 98	39	15	278		237	68	-	
"	TA 100	131	136	TNTC	TNTC	TNTC	TNTC	+	
"	TA 1535	14	18		TNTC			+	
"	TA 1537	10	5			77	43	+	
"	TA 1538	19	12	290		151	33	+	

(a) + = expected result, - = unexpected result (see discipline note)

TA 98 showed an unexpected low response to DMBA.

TABLE 2-B

POSITIVE CONTROL REVERTANT RATE

Date	Strain	Spontaneous Rev		AF	MNNG	BP	DMBA	Re-sponse (a)	Init
		S-9	No S-9	S-9	No S-9	S-9	S-9		
27 May	TA 98	23	30	TNTC		215	179	+	
"	TA 100	132	130	TNTC	TNTC	TNTC	TNTC	+	
"	TA 1535	6	6		TNTC			+	
"	TA 1537	6	3			106	33	+	
"	TA 1538	17	7	TNTC		148	18	-	

(a) + = expected result, - = unexpected result (see discipline note)

TA 1538 did not react as expected to DMBA.

TABLE - 3

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

2 May 80

Strain No.	Histidine (a) Requirements	Ampicillian (b) Resistance	uvr=B (c) Deletion	rfa Crystal Violet (d)	Sterility Control (e
TA 100	+	+	+	16mm	+
TA 1537	+	21mm	+	+	+
WT	NT	NT	NT	NT	NT
Diluent	NT	NT	NT	NT	NT
Test Compound (s)					
#1 N-Hexyl-2-oxazolidone	NT	NT	NT	NT	+
#2 N-octyl-glutarimide	NT	NT	NT	NT	+
#3_____	NT	NT	NT	NT	
#4_____	NT	NT	NT	NT	
#5_____	NT	NT	NT	NT	

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irratiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; WT= wild type.

Spontaneous Revertants

Strain	Average	Range					Average
TA 100	160	120-200	with S-9	99	81	77	86
			NO S-9	93	77	94	88

Test Inoculated By: F. Pulliam Date: 2 May 80

Test Read By: F. Pulliam Date: 4 May 80

TABLE - 4

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) N-Hexyl-2-oxazolidone (2) _____

(3) _____ (4) _____ (5) _____

Date: 2 May 80 Performed by: Pulliam, Summers

Substance dissolved in: (1) ETOH (2) _____ (3) _____

(4) _____ (5) _____

Visual estimation of background lawn on
Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
0.1 NO S-9	Toxic	Toxic	Toxic	Toxic	
0.01	Toxic	Toxic	Toxic	Toxic	
0.001	75	60	56	64	
0.0001	93	93	79	88	
0.1 with S-9	Toxic	Toxic	Toxic	Toxic	
0.01	Toxic	Toxic	Toxic	Toxic	
0.001	83	82	85	83	
0.0001	55	56	58	56	

TABLE - 5A
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) N-Hexyl-2-oxazolidone (2) _____

(3) _____ (4) _____ (5) _____

Date: 20 May 80 Performed By: Pulliam, Summers

Substance dissolved in: (1) ETOH (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
1	0.001	TNTC	TNTC	TNTC	TNTC	242	TNTC	239	TNTC	252	TNTC
1	2×10^{-4}	TNTC	TNTC	TNTC	TNTC	TNTC	298	TNTC	272	TNTC	292
1	4×10^{-5}	86	TNTC	172	TNTC	56	337	48	TNTC	47	TNTC
1	8×10^{-6}	TNTC	87	TNTC	157	TNTC	75	TNTC	58	TNTC	TNTC
1	1.6×10^{-6}	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
1	3.2×10^{-7}	TNTC	105	TNTC	194	TNTC	101	TNTC	89	TNTC	139

TABLE - 5B
SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) N-Hexyl-2-oxazolidone (2) _____

(3) _____ (4) _____ (5) _____

Date: 27 May 80 Performed By: Pulliam, Summers

Substance dissolved in: (1) ETOH (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
1	0.001	23	24	111	106	7	10	4	4	6	10
1	2×10^{-4}	22	30	110	115	5	13	4	3	10	17
1	4×10^{-5}	69	100	101	109	11	9	7	6	15	16
1	8×10^{-6}	73	175	102	95	10	13	3	5	7	17
1	1.6×10^{-6}	81	135	117	168	8	13	4	6	10	14
1	3.2×10^{-7}	86	133	109	117	12	12	6	5	14	21

TABLE - 6
MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: N-Hexyl-2-oxazolidone Dissolved in: ETOH

Date: 27 May 80 Performed by: F. Pulliam

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
0.001	TA 98	*	0.04	8×10^{-6}	TA 1535	0.3	0.75
2×10^{-4}	TA 98	*	0.27	1.6×10^{-6}	TA 1535	0.15	0.75
4×10^{-5}	TA 98	1.69	2.6	3.2×10^{-7}	TA 1535	0.45	0.64
8×10^{-6}	TA 98	1.86	5.89				
1.6×10^{-6}	TA 98	2.21	4.34	0.001	TA 1537	0.16	*
3.2×10^{-7}	TA 98	2.42	4.26	2×10^{-4}	TA 1537	0.16	*
				4×10^{-5}	TA 1537	0.66	*
0.001	TA 100	*	*	8×10^{-6}	TA 1537	*	*
2×10^{-4}	TA 100	*	*	1.6×10^{-6}	TA 1537	0.16	*
4×10^{-5}	TA 100	*	*	3.2×10^{-7}	TA 1537	0.49	*
8×10^{-6}	TA 100	*	*				
1.6×10^{-6}	TA 100	*	*	0.001	TA 1538	*	*
3.2×10^{-6}	TA 100	*	*	2×10^{-4}	TA 1538	0.36	*
				4×10^{-5}	TA 1538	0.96	*
0.001	TA 1535	*	0.43	8×10^{-6}	TA 1538	*	*
2×10^{-4}	TA 1535	*	0.86	1.6×10^{-6}	TA 1538	0.36	*
4×10^{-5}	TA 1535	0.38	0.32	3.2×10^{-7}	TA 1538	0.84	0.23

* MUTAR value was insignificant

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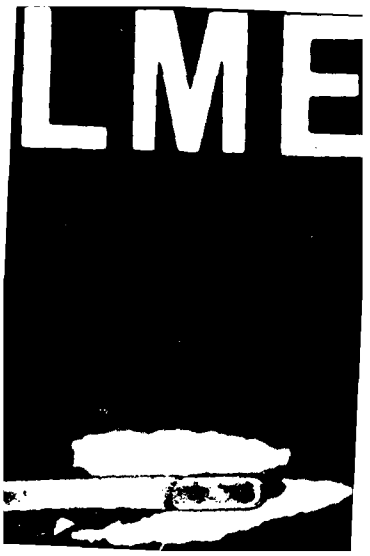
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